PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
11345(PCT)		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IN 00/00099	11/10/2000	13/10/1999
Applicant		
AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IN 00/00099

A. CLASSIFICATION OF SUBJECT MATT[redacted]
IPC 7 C07K14/415 C0[redacted]/81 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PATELL V.M. ET AL.: "Oryza sativa Variety IR64 (cDNA clone AGTSAL-11 from 7 days old seedlings)" EMBL DATABASE ENTRY AF192975; ACCESSION NO. AF192975, 9 November 1999 (1999-11-09), XP002169663 cited in the application	1-10
A	MOONS A. ET AL.: "MOLECULAR AND PHYSIOLOGICAL RESPONSES TO ABSCISIC ACID AND SALTS IN ROOTS OF SALT-SENSITIVE AND SALT-TOLERANT INDICA RICE VARIETIES" PLANT PHYSIOLOGY, vol. 107, 1995, pages 177-186, XP000983692 ISSN: 0032-0889 page 180, column 2, paragraph 3 -page 184, column 1, paragraph 3	1-11

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2001	28/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schönwasser, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]IN 00/00099

(continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BAEK J.-M. ET AL.: "Nucleotide sequence homology of the cDNAs encoding soybean Bowman-Birk type proteinase inhibitor and its isoinhibitors" BIOSCIENCE, BIOTECHNOLOGY AND BIOCHEMISTRY, vol. 58, no. 5, 1994, pages 843-846, XP002123337 ISSN: 0916-8451 the whole document	9

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 May 2001 (03.05.2001)

PCT

(10) International Publication Number
WO 01/30990 A2

(51) International Patent Classification[7]: C12N 15/00

(21) International Application Number: PCT/IN00/00099

(22) International Filing Date: 11 October 2000 (11.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
997/MAS/99 13 October 1999 (13.10.1999) IN

(71) Applicant *(for all designated States except US)*: **AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD.** [IN/IN]; "Sunbeam" 106 Prenderghast Road, Secunderabad - 500 003, Andhra Pradesh (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PATELL, Villo, Morawala** [IN/IN]; "Sunbeam" 106 Prenderghast Road, Secunderabad - 500 003, Andhra Pradesh (IN). **ANTONY, Chettoor, Mathai** [IN/IN]; "Sunbeam" 106 Prenderghast Road, Secunderabad - 500 003, Andhra Pradesh (IN). **CHANDRAN, Divya** [IN/IN]; "Sunbeam" 106 Prenderghast Road, Secunderabad - 500 003, Andhra Pradesh (IN). **MADURAPPA, Ashok** [IN/IN]; "Sunbeam" 106 Prenderghast Road, Secunderabad - 500 003, Andhra Pradesh (IN).

(74) Agents: **ANAND, Pravin** et al.; Anand and Anand, Advocates, B-41, Nizamuddin East, New Dehli 110 013 (IN).

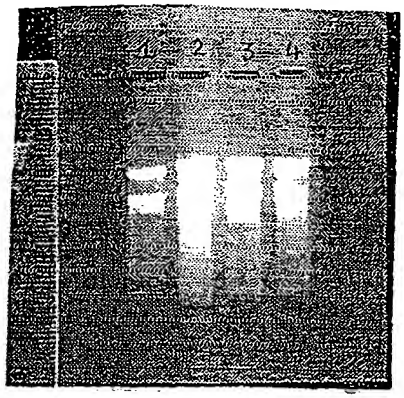
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

[Continued on next page]

(54) Title: ISOLATED NUCLEIC ACID SEQUENCE CONFERRING SALT TOLERANCE IN RICE PLANT



Lanes : 1 IR64 S-S 10 µL. 2 IR64C 10 µL. 3 RH51 S-S 10 µL. 4 KDS-S 10 µL

(57) Abstract: The present invention relates to an isolated nucleic acid sequence AGT-SAL 11 encoding polypeptides which confers salt tolerance on plants and other organisms.

WO 01/30990 A2

Copy for the designated Office (DO/US)
09/868025

PCT/IN00/00099

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

ANAND, Pravin
Anand and Anand, Advocates
B-41, Nizamuddin East
New Dehli 110 013
INDE

Date of mailing (day/month/year) 18 September 2001 (18.09.01)	
Applicant's or agent's file reference 11345(PCT)	**IMPORTANT NOTIFICATION**
International application No. PCT/IN00/00099	International filing date (day/month/year) 11 October 2000 (11.10.00)
International publication date (day/month/year) 03 May 2001 (03.05.01)	Priority date (day/month/year) 13 October 1999 (13.10.99)
Applicant AVESTHA GENGRAINE TECHNOLOGIES PVT. LTD. et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
13 Octo 1999 (13.10.99)	997/MAS/99	IN	16 Marc 2001 (16.03.01)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

David MALEK

Telephone No. (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 11345(PCT)

Box No. I TITLE OF INVENTION

"ISOLATED NUCLEIC ACID SEQUENCE CONFERRING SALT TOLERANCE IN RICE PLANT."

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD.
"Sunbeam" 106 Prenderghast Road,
Secunderabad - 500 003
Andhra Pradesh
India

☐ This person is also inventor.

Telephone No. 0091 80 3532060

Facsimile No. 0091180 3436404

Teleprinter No.

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PATELL Dr. Villo Morawala
"Sunbeam" 106 Prenderghast Road
Secunderabad - 500 003
Andhra Pradesh
India

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ANAND, Pravin; KUMAR, Shanti;
SHANKER, Archana; BHATNAGAR, Jaya
ANAND & ANAND, Advocates
B-41, Nizamuddin East,
New Delhi - 110 013
India

Telephone No. 0091 11 4645076

Facsimile No. 0091 11 4624243

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Sheet No. 2

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ANTONY Chettoor Mathai
"Sunbeam" 106 Prenderghast Road,
Secunderabad - 500 003,
Andhra Pradesh,
India

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CHANDRAN Divya
"Sunbeam" 106 Prenderghast Road,
Secunderabad - 500 003,
Andhra Pradesh
India

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MADURAPPA Ashok
"Sunbeam" 106 Prenderghast Road,
Secunderabad - 500 003,
Andhra Pradesh,
India

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: IN

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus .....
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China .....
☒ CR Costa Rica .....
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ DM Dominica
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia
☒ HR Croatia .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☐ IN India .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan .....
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco .....
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ NO Norway
☒ NZ New Zealand .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia .....
☒ SK Slovakia .....
☒ SL Sierra Leone .....
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
☒ TZ United Republic of Tanzania
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America .....
☒ UZ Uzbekistan .....
☒ VN Viet Nam .....
☒ YU Yugoslavia .....
☒ ZA South Africa .....
☒ ZW Zimbabwe .....

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....
☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 13 October 1999 (13.10.1999)	997/Mas/99	India		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

(/ EPO

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following number of sheets:

request	:	4
description (excluding sequence listing part)	:	17
claims	:	1
abstract	:	1
drawings	:	
sequence listing part of description	:	
Total number of sheets	:	23

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☒ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract:

***Language of filing of the international application:** English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

For each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

(Archana Shanker)IN/PAT 149

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: 11345(PCT)

Applicant: AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . Rs. 5000.00 [T]

2. SEARCH FEE . . . . . USD 231.25 [S]

International search to be carried out by EPO

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains 23 sheets.

first 30 sheets . . . . . USD 427.00 [b1]

____ remaining sheets x ____ additional amount = [b2]

Add amounts entered at b1 and b2 and enter total at B . . . . USD 427.00 [B]

Designation Fees

The international application contains 108 designations.

92 number of designation fees payable *(maximum 8)* x 8 amount of designation fee = USD 736.00 [D]

Add amounts entered at B and D and enter total at I . . . . . USD 1163.00 [I]

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . Rs. 3000.00 [P]

5. TOTAL FEES PAYABLE . . . . . Rs. 8000.00 + USD 1394.25 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

[X] The designation fees are not paid at this time.

MODE OF PAYMENT

- [ ] authorization to charge deposit account (see below)
- [X] cheque
- [ ] postal money order
- [ ] bank draft
- [ ] cash
- [ ] revenue stamps
- [ ] coupons
- [ ] other *(specify)*:

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ ____ [ ] is hereby authorized to charge the total fees indicated above to my deposit account.

[ ] *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

[ ] is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account No. | Date *(day/month/year)* | Signature

See Notes to the fee calculation sheet

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

IN 00/99

4

THE PATENTS ACT,1970

It is hereby certified that hereto is a True Copy of the Provisional and Complete Specification and drawings filed in respect of Patent Application No.997/MAS/1999,dated October,13th 1999 by Avesthagen Graine Technologies Private Limited, an Indian Company of "Sunbeam", 106 Prenderghast Road, Secunderabad- 500 003,Andhara Pradesh, India, ...

09786825

..... In witness thereof
I have hereunto set my hand

Dated this the 6th . day of December 2000
15th day of Agrahayana,1922(SAKA)

S. Chandrasekaran

(S. CHANDRASEKARAN)
DEPUTY CONTROLLER OF PATENTS AND DESIGNS.

TEN CE BRANCH,
ENN 090.

THE PATENTS ACT, 1970

PROVISIONAL SPECIFICATION

Section 10

"Cloning and Sequencing of AGTSa1 11 Rice Gene from IR-64 Variety Implicated in Salinity Stress Tolerance"

Avesthagen Graine Technologies Private Limited an Indian company of "Sunbeam", 106 Prenderghast Road, Secunderabad-500 003, Andhra Pradesh, India

The following specification particularly describes and ascertains the nature of this invention:

CLONING AND SEQUENCING OF AGTSal-11 RICE GENE FROM IR-64 VARIETY IMPLICATED IN SALINITY STRESS TOLERANCE

Area of invention:
Altered gene expression lies at the heart of the regulatory mechanisms that control cell biology. Comparisons of gene expression in different cell types provide the underlying information we need to analyze the biological processes that control our lives. Effective methods are needed to identify and isolate those genes that are differentially expressed in various cells or under altered conditions. mRNA Differential Display Technology developed by Drs.Arthur.B.Pardee and Pen Liang at Harvard Medical School is a powerful new tool for identifying and cloning differentially expressed genes.

What is known till date:
technique is inefficient for low abundant transcripts. Only one way comparisons are possible and it Subtractive hybridization was used for identifying and cloning differentially expressed mRNAs. The basic principle of subtractive hybridization involves the hybridization of cDNAs from one population in which mRNAs are differentially expressed to excess constitutively expressed cDNAs from another population. The sequences that are common to both populations are removed using hydroxyapatite chromatography, avidin-biotin binding or oligo-dT beads.

Problems encountered with subtractive hybridization:
Despite the enormous success of subtractive hybridization techniques in cloning different genes, this requires multiple subtraction steps. Therefore a new strategy was developed which permits exponential amplification of cDNAs that differ in abundance, whereas amplification of sequences with identical abundance in 2 populations is suppressed.

Novelty of Differential Display Technique:
Differential Display is a powerful tool for analyzing gene expression, allowing genes to be isolated solely on changes in phenotype and without prior knowledge of protein or nucleic acid sequence. This new technique is a flexible and comprehensive method for detecting almost all genes expressed in a particular cell and for identification of differences in gene expression between different cell types in both mammal and plant systems. There is simultaneous display of all up and down regulated genes; it permits side-by-side comparisons of mRNA from different sources; only a few (g of RNA is required, compared to 50X or more for subtractive hybridization; highly reproducible; And finally high speed of analysis.

The Differential Display technique:
This method involves the reverse transcription of the mRNAs with oligo-dT primers anchored to the beginning of the poly (A) tail, followed by the Polymerase Chain Reaction in the presence of a second 10mer, arbitrary in sequence. The amplified cDNA sub-populations of 3' termini of mRNAs as defined by this pair of primers are distributed on a DNA sequencing gel and visualized by autoradiography. Each pair of primer produces a distinct pattern of bands. The band pattern obtained with each primer is compared. Differentially expressed bands are cut out of the gel and the DNA is eluted and re-amplified. The amplified products are cloned into suitable vectors and their sequence deduced.

Scope of invention:
In this project we set out to ask the question- what makes a resistant rice variety resistant and a susceptible variety susceptible in response to salt Stress? Two Indian varieties of rice- IR-64 and RASI were taken as samples for

the experiment. While IR-64 is susceptible to high sal[redacted] stress, RASI is resistant to the same. Therefore this Differential Di[redacted]y technique can be used to study the regulat[redacted]of gene expression at the cellular level in these two varieties under salt stress conditions and possibly isolate the genes responsible for susceptibility or resistance in IR-64 and RASI respectively. Appropriate control were maintained which consisted of rice plants not subjected to salt stress but were of the same age.

Method:

The IR-64 and RASI seeds were subjected to salt stress using 150mM NaCl. The RNA was isolated from both stressed plants and unstressed controls. Further processing of the RNA was done following the protocol provided by Gen-Hunter's Differential Display kit. The RNA was reverse transcribed using H-T11 primers to obtain the cDNA. This DNA was amplified by PCR using H-T11 primers and an arbitrary primer H-AP1. The PCR products were resolved on a 6% denaturing polyacrylamide gel and subjected to autoradiography. The autoradiogram showed 54 differentially expressed bands. The band labeled A-11 was cut out from the gel and DNA eluted. Reamplification of the DNA was done using the same primer set and PCR conditions. The PCR fragment was cloned into TOPO TA cloning vector, which is unique, fast and efficient way to clone PCR products. The vectors are linearized, have an extra 3'T overhang and are activated with topoisomerase. Ligation takes advantage of the template-independent addition of a single adenosine (A) to the 3' end of the PCR products by Taq DNA Polymerase. The positive clones were checked for presence of insert by digesting with Eco RI restriction endonuclease. Two clones showing insert release were subjected to sequencing using sequences of the vector that flank the insert sites as primers; M13 forward and reverse primers were used for this purpose. the sequencing results using M13 reverse primer shows that the sequence of interest lies between nucleotides 130 and 310 which ends with a stretch of poly A's. since the fragment of interest was amplified using specific oligo-dT primers, its position in the sequence was located by searching for a poly A stretch downstream. This stretch was found around position 310, indicating the 3' end of the sequence of interest. A blast search was done using this sequence to find homology with gene sequences in different databases. The nucleotide sequence with the highest homology to the A11 fragment (submitted as query sequence) was found to be Rice root Oryza sativa cDNA clone, showing almost 100% identity and an 'e' value of -82. The gaps were found to be none, further emphasizing that the sequence obtained is of an authentic rice gene. Thus the sequence of the 3'end of the gene was established. (~ 180 base pairs long). The total gene was obtained by doing a PCR on the genomic DNA extracted from rice calli using forward and reverse primers specifically designed- ANT DD2F and ANT GSPI DD2 respectively. The PCR products were cloned and then sequenced to give the rest of the gene sequence. (5' end). The different sequences obtained were subjected to multiple alignment to give the final complete sequence of the gene- AGTSal-11.

Final Sequence of gene AGTSal-11: (520 base pairs long)

5'- ATGGCAGCAAACTCCATCTTGGGGTGTGGCGTGAGCACACCAAGAAATTCTCCCCTCAGTGGTTTGCAGCTGTCCA
TGCCGCTGTTCCATTCATTGGAATGCTGAGGAAATCTGTCAACATGCCCAAGACTGCCATGGCATTCACCATAGCAG
CCTCCATTGTTGGTCAGACAATCGGGTCGAGGGCGGAGCGCATTCGTCTGAAGGCACTGGCTGCAAAGAGCGACGCT

GCTCGCGATGAAGATGATGGCGGGACGGGCTTCTGGTGGTGCTGCTGCTCCAACACCAAGCATGTGTTTCTGATTGC
TCACTGATTGGAAAATTTGTATCTACCAGTATCCCTGGAGAGTGGAGAGTTGATATTGAGTCTATTTTATCTTGTGA
TGTAATTGCCTTTGCTTGTCCCTC[redacted]AAGTATTCGTTTGTTTGTGGGATGAGACAA[redacted]GGAATAAGAGTGCTACTAT
ATACACGATCATTCTGTTGTTAA[redacted]TGCCAGTTCTGCAGTTCATGTATCTGTAA[redacted]ATGATGCTGGATTTCTAC
TATTTATCAATCGTCATTATACTG[redacted]GTGTAAAAAAAAAAAA-3'

Potential role of the gene AGTSal-11 in salinity stress:
Since this gene was found to be over expressed in IR-64 stressed plants as compared to the IR-64 control plants, it could have a potential role in salinity stress. Hence overproduction of the protein product of the gene may result in increased tolerance to salt stress of transgenic rice plants. Genetically engineered crop plants that overproduce the gene product might thus, acquire the ability to tolerate high salinity.

TTTACCTTGCCTGCTCGGATGGCAGCAAACTCCATCTTGGGGTGT
GGCGTGAGCACACCAAGAAAT
TCTCCCCTCAGTGGTTTGCAGCTGTCCATGCCGCTGTTCCATTCAT
TGGAATGCTGAGGAAATCTG
TCAACATGCCCAAGACTGCCATGGCATTCACCATAGCAGCCTCCA
TTGTTGGTCAGACAATCGGGT
CGAGGGCGGAGCGCATTCGTCTGAAGGCACTGGCTGCAAAGAGC
GACGCTGATTCCACCACCGTGG
CTGACATGTATCCAAACAAGACTGCAAATTGCAGTGACACCGAG
GGCAAGGCATGGGATCCGCTCG
CGATGAAGATGATGGCGGGACGGGCTTCTGGTGGTGCTGCTGCTC
CAACACCAAGCATGTGTTTCT
GATTGCTCACTGATTGGAAAATTTGTATCTACCAGTATCCCTGGA
GAGTGGAGAGTTGATATTGAG
TCTATTTTATCTTGTGATGTAATTGCCTTTGCTTGTCCCTCAGAAG
TATTCGTTTGTTTGTGGGAT
GAGACAAGTGGAATAAGAGTGCTACTATATACACGATCATTCTGT
TGTTAAGTTTGCCAGTTCTGC
AGTTCATGTATCTGTAATTTGATGATGCTGGATTTCTACTATTTAT
CAATCGTCATTATACTGTGT
GTAAAAAAAAAAA

Dated this 12th day of October 1999

Archana Shanker
Of Anand & Anand, Advocates
Agents for the Applicants

Form 2

THE PATENTS ACT 1970

COMPLETE SPECIFICATION

SECTION 10

1971/MAS/99
13-10-1999.

"Isolated nucleic acid sequence conferring salt tolerance in rice plant"

ORIGINAL

Complete After Provisional
Left 11 OCT 2000

Avesthagen Graine Technologies Private Limited, an Indian Company of "Sunbeam", 106 Prenderghast Road, Secunderabad –500 003 , Andhra Pradesh, India

The following specification particularly describes and ascertains the nature of this invention and the manner in which it is to be performed:

Field of the invention

The present invention relates to an isolated nucleic acid sequence conferring salt tolerance in rice plant. More specifically this invention relates to a method for conferring salt tolerance in plants.

Altered gene expression lies at the heart of regulatory mechanisms that control cell biology. Comparisons of gene expression in different cell types provide the underlying information that analyzes the biological processes that control our lives. Effective methods are needed to identify and isolate those genes that are differentially expresses in various cells or under altered conditions.

Background

Life can not exist without water. It forms an important constituent of the plant and animal cell and is present to the extent of 80 to 90%. Water is essential for plants due to the following reasons :

1. It is the major component of protoplasm. If the protoplasm is dehydrated, it ceases to be active and the protoplasm looses its physical and chemical properties. Water maintains turgidity of cells.

2. Water is a universal solvent. The intake of minerals and nutrients from the external medium into the cell is only in the dissolved form.

3. Water serves as the medium for translocation of minerals from the soil to leaves through the xylem and food manufactured by the leaves to other plant parts via phloem.

4. Water also plays an important role in the transport of plant hormones.

5. Plant movements (especially of certain organs) are caused by changes in water content of cells.

6. Water is directly involved in the bio-chemical reactions that take place in plant cells. Hydrolyis of macromolecules takes place by the addition of water. Water is the source of hydrogen for the reduction of carbon-dioxide during photosynthesis. Water is one of the products of cellular respiration. All these reactions are influenced by the availability of adequate and good quality water.

Since water plays such an important role in plants, its deficit severely effects cellular functions, plant growth & development and reduces yields . However the plant devises a number of changes that occur at the whole plant, physiological, cellular, bio-chemical and molecular levels in an attempt to cope with moisture stress.

Furthermore, due to the widespread use of irrigation and limited water supply, many cultivated areas have become increasingly salinized. Irrigation imparts increasingly salt concentration when the available irrigation water evaporates and leaves previously dissolved salts behind.

Dissolved salts in the soil increase the osmotic pressure of the solution in the soil and tends to decrease the rate at which water from the soil will enter the roots. If the solution in the soil becomes too saturated with dissolved salts, the water may actually be withdrawn from plant roots. Thus the plants slowly starve though the supply of salts and dissolved nutrients may be more than ample.

Salinity and water deficit have shown to induce the expression of number of genes. These gene products have either regulatory role in gene expression or a functional role in adaptive responses of plant cells to the stress.

Salinity refers to the presence of various salts in soil and irrigation water in concentrations that affect the growth and yield of plants. Sodium chloride (common salt), is often the dominant salt present in saline soils. Saline-alkaline and sodic soils may have excess of chlorides, sulphates and bicarbonates of sodium, calcium and potassium in addition to other inorganic ions.

Saline soils have a soil water conductivity of 4 deci-seimen/meter and exchangeable sodium percentage of not less that 15. This translates into

nearly 2.56 g/L of total dissolved salts in an extract or if all the salt is NaCl, an ionic concentration of 44.14 mM.

The irrigation water in majority of the rice growing areas is generally of marginal or poor quality (EC of 2-5ds/m or more). Though water is present it is unavailable to plants because the osmotic potential of soil is altered. To exclude salts and minimize ion toxicity, water must be imported against a free energy gradient. However, if water is taken up freely, the endogenous salt concentration rises.

Macromolecular assemble and enzyme activity associated with shaping and maintaining each cell can proceed only with a properly constituted ionic environment. The inorganic ions selectively neutralize charges on macromolecular surfaces and simultaneously permit formation of intramolecular bridges that determine the final conformation of many proteins. The same ions also determine the availability of free water around enzymes and their substrates and thus the rate of catalysis. Finally, ionic gradients, set up at considerable cost to the plant cell, constitute free energy gradients that can be tapped to direct the flow of organic molecules and between cells [Claes et al., 90].

An extracellular ion excess invariably disrupts the ionic balance intracellularly. With the influx of salt, proteins may denature or aggregate leading to a loss of function, gradient-driven pumps may reverse and thus block the normal redistribution of symported molecules , membrane fluidity and consequently, the activity of some membrane components may change, and even the entry of water may be restricted . Some ions may have additional secondary effects. For example, increasing amounts of intercellular Na+ can lead to decreases in the concentration of K+ [Ben-Hayyim et al., 1987; Binzel et al., 1988]. This, in turn, reduces the rate of photosynthesis[Pier and Berkowitz, 1987], and , based on studies with bacteria can accelerate polysome decay and degradation of the free ribosomal proteins [St. John and Goldberg, 1980]. Salt imposed stress has been shown to have an impact even before ions enter the cell. Extracellular Na+ (or mannitol), for example, can leach Ca^{2+} from root cell plasmalemma, and as a result of membrane destabilization, increases K+ efflux[Cramer et al., 1985].

These are only the immediate problems facing the cell. If the stress is prolonged, normal maintenance processes are impaired because general

protein synthesis [Hurkman and Tanaka, 1987] and metabolism [Criddle et al., 1989] both decline. Denatured proteins may form inactive complexes with otherwise functional proteins. Enzymes may be poisoned when inorganic cofactors are displaced by incoming salts.

Rice is a salt sensitive plant and the most important cereal crop of the world. This crop is grown in diverse ecosystems and extensively in the tropics. Rice is the staple food of majority of the people in South & East Asian nations, parts of South America and Africa. The present production of rice in the world falls well short of the demand. To meet the ever-increasing demand, continuous improvement in the quality and productivity of this cereal is vital.

Several biotic and abiotic factors are important constraints in increasing the quality and yield of rice. Biotic stresses in the form of pests and diseases considerably affect rice productivity. Abiotic stresses however have been shown to cause more harm to the rice crop than biotic stresses. The major abiotic stresses, which significantly hamper rice yields are drought, salinity, floods, extremes of temperature and metal toxicity.

Minimizing crop losses by abiotic stresses especially drought and salinity is an important area for the overall improvement of rice. A thorough understanding of the responses of the rice plant to abiotic stresses is fundamental for developing a strategy to make the rice plant more hardy.

Initial work in understanding the effects of abiotic stress on rice was done at the whole plant level. The role, interactions and alterations of root-shoot characteristics in response to stress in rice has been studied. Later work focused on the physiology of stress. The effects of drought and salinity on the physiological processes like metabolism, growth and development has come forth.

Efforts have also been made to improve the performance of rice crop under limiting environmental conditions through traditional breeding programs and agronomic practices. Strategies for the evaluation of rice for drought and salinity tolerance using field screening and multi-location testing have been developed. These approaches have also been able to distinguish rice varieties into susceptible and tolerant ones. The development of molecular linkage maps and the use of molecular markers, of late, is helping selection and breeding for drought resistance. Molecular

markers linked to root traits, osmotic adjustment and other stress tolerant characters are now being identified and used for selection and breeding.

The molecular responses of plants to abiotic stresses is a complex phenomenon. However, advances in molecular biology offer new tools to investigate changes in plants, at the cellular and molecular level, in response to abiotic stresses.

Relatively speaking, this species is more sensitive to salt stress at the seedling stage and the reproductive stage [Lutts et al., 1995]. Excess salt leads to reduced seed germination and poor seedling vigor. During the vegetative phase, premature senescence of leaves and reduced number of tillers can occur. During the reproductive stage, the number of spikelets per panicle get significantly reduced [Lutts et al., 1996]

Furthermore, rice cultivation in tropical areas is mostly dependent on seasonal rainfall, vagaries of tropical monsoon renders the growth and yield of rice crop uncertain. The modern high yielding varieties of rice in particular are unable to attain their full genetic potential in the absence of adequate and good quality water.

Drought occurs when there is insufficient soil water to be taken up by the plants over a period of time to meet its transpirational requirements. Sustained drought results in complete loss of free water and will result in desiccation and dehydration. Concentration of solutes in the cell leads to drop in cellular water potential. Loss of turgor leads to changes in the cell volume and membrane area. The crucial cell wall plasma membrane continuum is lost. An osmotic shock can cause extensive cell damage through disruption of membrane integrity and leakage of cellular contents. Cellular water deficit causes extensive damage to functional proteins and increases formation of misformed proteins. Impairment in the normal metabolic pathways leads to formation of toxic and highly reactive by products such as the reactive oxygen species. Many other cellular changes similar to those occuring during salt stress are also observed during drought.

In Rice, at the plant level, drought affects several developmental processes. Seed germination is non-uniform. At the Vegetative stage, canopy photosynthetic rates decrease drastically. Root growth is affected. Leaf rolling and leaf scorching is observed. At the reproductive stage, drought causes pollen sterility, small, thin and deformed anthers. Drought during

anthesis causes inhibition of another dehiscence and pollen germination, reduced pollen viability, failure of the panicle to exert the flag leaf, resulting in loss of grain set. Water constraint during ripening causes incomplete grain filling [O'Toole and Moya, 1981].

Molecular Responses of Rice to Salinity and Moisture stress.

Osmotic stress (such as salinity and Drought) leading to water deficit elicit complex molecular responses in plants. The events described here are common to all plants and also apply to Rice.

The molecular responses of plants to water deficit is dependent upon the type of stress(salinity/drought), severity of stress (mild/moderate or severe) and duration of stress (sporadic or chronic). A gradual onset of stress allows cellular mechanisms to adopt better while a sudden severe stress results in cellular damage and activates repair mechanisms. Plant factors such as genotype/variety, developmental stage (seed/seedling/vegetative or reproductive stage) and organ (root/shoot etc.) exposed to stress also influences the nature of response [Bray, 1997].

Molecular events during water deficit has been investigated using four major approaches [reviewed in Ingram and Bartels, 1996] :

1. Examining tolerant systems such as seeds and resurrection plants.
2. Analyzing mutants from genetic model species.
3. Analyzing the effects on agriculturally relevant plants.
4. By the targetted expression of drought related genes in vivo using transgenic plants.

The responses of plants to water deficit at the molecular level normally occur in the following sequence [Bray, 1993}"

1. Cellular perception of the stress.
2. Signal transduction events.
3. Alterations in the gene expression.
4. The role of gene products induced by salinity and drought in stress avoidance of tolerance.

The Role of Gene Products induced by Salinity and Drought.

Salinity and Water deficit have shown to induce the expression of a number genes. These gene products have either a regulatory role in gene expression or a functional role in the adaptive responses of plant cells to the stress.

Many genes have been identified and characterized to have a definite role in the response of plants to salinity and drought, and are induced by a complex mechanism of stress perception and signal transduction events. Stress related gene products have a role in moisture stress tolerance such as signaling molecules, regulatory proteins, protection of cellular structures, synthesis of osmoprotectants, ion sequestration, chaperon activity and protein stabilization, protein degradation, scavenging of accumulated toxins(especially reactive oxygen species), promotion of damage repair mechanisms, anti-pathogen activity and others.

Changes, in the tissue specific gene expression, are fundamental to the responses that occur during salinity and drought and influence many of the short and long term cellular changes that determine stress resistance or susceptibility. Northern Blot analysis, using stress related cDNA probes, offers a simple but powerful tool to monitor alterations in gene expression in roots and shoots, in response to salinity and water deficit, while comparing a susceptible and tolerant variety.

Furthermore, subtractive hybridization technique has been used for identifying and cloning differentially expresses mRNAs. The basic principle of subtractive hybridization involves the hybridization of cDNAs from one population in which mRNAs are differentially expressed to excess constitutively expressed cDNAs from another population. The sequence that are common to both the populations are removed using hydroxypatite chromatography, avidin –biotin binding or oligo- dT beads. Despite the enormous success of subtractive techniques in cloning different genes, this requires multiple subtraction steps. Therefore, a new strategy was developed which permits exponential amplification of cDNAs that differ in abundance in 2 populations is suppressed.

Differential display is also a power tool for analyzing gene expression, allowing genes to be isolated solely on changes in phenotype and without prior knowledge of protein or nucleic acid sequence. This

technique is flexible and is a comprehensive method for detecting almost all genes expressed in a particular cell and for identification of differences in gene expression between different cell types in both mammal and plant systems. There is a simultaneous display of up and down regulated genes, it permits side-by –side comparisons of mRNA from different sources; only a few (g of RNA is required, compared to 50X or more for subtractive hybridization; highly reproducible and finally high speed of analysis.

This method involves the reverse transcription of the mRNA with oligo-dT primers anchored to the beginning of the poly (A) tail, followed by the polymerase chain reaction on the presence of a second 10mer, arbitrary in sequence. PCR primers and conditions are chosen such that any given reaction yields a limited number of amplified cDNA fragments permitting their visualization as discrete bands following Gel Electrophoresis. The amplified cDNA sub-populations of 3' termini of mRNAs as defined by this pair of primers are distributed on a DNA sequencing gel and visualized by autoradiography. Each pair of the primer produces a distinct pattern of bands. The band pattern obtained with each primer is compared. Differentially expressed bands are cut out of the gel and the DNA is eluted and re-amplified. The amplified products are cloned into suitable vectors and their sequence deduced.

The prior art for our experiments includes interalia the work done by scientists in relation to rice and proteinase inhibitors discovered from rice under biotic conditions.

Also the prior art known in this field addressed only biotic stress (host-pathogen interaction), whereas our invention addresses a novel issue that is the area of abiotic stress(salinity stress).

Summary of the invention

The object of the present invention is to correlate the expression pattern (at the mRNA levels) of genes under study with their role in abiotic stress tolerance or susceptibility in IR64 (susceptible variety) and RASI (tolerant variety).

Yet another object of the present invention is to compare the differences in the expression of genes encoding stress proteins during salinity and desiccation.

Further object of this invention is to assess the gene expression pattern in root and shoot during different stages of salt and dehydration.

To achieve the said objects, the present invention relates to relates to a nucleic acid sequence comprising a polynucleotide, AGT- SAL 11 having a sequence SEQ.ID No.1.

The AGT-SAL11 polynucleotide sequence encodes a polypeptide as shown in SEQ ID No. 2.

The polynucleotide sequence is a full length AGTSAL 11 gene. The said polypeptide has bi-functional units.The said polypeptide has glycosylation and phosphorylation sites. Said glycosylation is O glycosylation.

Said AGT-SAL 11 has a mixture of $\mapsto\updownarrow$ type of secondary structure.

Said polypeptide has similarity with proteinase inhibitors of Bowman Birk type II of super family of proteinase inhibitors.

The present invention further relates to a method for conferring salt tolerance on a plant, the method comprising introducing into the plant a recombinant expression cassette comprising a plant operator operably linked to AGT-SAL 11 polynucleotide sequence.

The invention has use over a broad range of types of plants and organisms. Such plants *interalia* includes cotton, maize, rice, soybeans, sugar beet, wheat, fruit, vegetables and vines. The major use of proteinase inhibitors is against biotic stress response such as bacterial, fungal, pest resistance etc. in plants. It is also useful in the treatment of cancer, HIV and other areas in the animal systems. The gene may be useful for food processing and enzyme industries as an inhibitor of proteinase activity as a biological preservative.

Detailed description of the invention

Two Indian varieties of rice IR64 and RASI were taken. While IR-64 is susceptible to high salt stress, RASI is resistant to the same. The differential display technique was used to determine the regulation of gene expression at the cellular level in these two varieties under salt stress

conditions and isolate the genes responsible for susceptibility or resistance in IR-64 and RASI respectively.

IR-64 and RASI seeds were subjected to salt stress using 150mM NaCl. The RNA was isolated from both stressed plants and unstressed controls. Further processing of the RNA was done following the protocol provided by Gen- Hunter's differential display kit. The RNA was reverse transcribed using H-T11 primers to obtain the cDNA. This DNA was amplified by PCR using H-T11 primers and an arbitrary primer H-API. The PCR products were resolved on a 6 % denaturing polyacrylamide gel and subjected to autoradiography. The autoradiogram showed 54 differentially expressed bands. The band labeled A-11 was cut out from the gel and DNA eluted. Reamplification of the DNA was done using the same primer set and PCR conditions.

The PCR product of AGT-SAL was cloned into TOP TA cloning vector, which is a unique, fast and an efficient way to clone PCR products. The vectors are linearized having an extra 3'T overhang and are activated with topoisomerase. Ligation takes advantage of the template independent addition of a single adenosine (A) to the 3' end of the PCR products by Taq DNA Polymerase. The positive clones were checked for the presence of insert by digesting with EcoRI restriction endonuclease.

Two clones showing insert release were subjected to sequencing using sequences of the vector that flank the insert sites as primers; M13 forward and reverse primers shows that the sequence of interest lies between nucleotides 130 and 310 which ends with a stretch of poly A's. Since the fragment of interest was amplified using specific oligo-dT primers, its position in the sequence was located by searching for a poly A stretch downstream. This stretch was found around position 310, indicating the 3' end of the sequence of interest.

For expressing the vector, the gene AGTSAL-11 (Accession No. AF 192975) should be cloned in expression vector where the protein of interest would be induced under inductive condition. There are so many vectors being used for this purpose, which ideally contain artificial ribosome binding site, transcription start site, transcription terminator, inducible promoter and a multiple cloning site (MCS) for cloning of desired gene at a particular site and a module for purification of he protein in the induced state. For the purification of protein of interest under inducible condition there are several

criteria that can be used such as GST (Glutathion S transferase) fusion protein where protein of interest can be purified by Glutathion affinity column and further the protein can be obtained by the treatment of endopeptidase with GST peptide specificity. The other popular protein expression has 6 X His tag which is coded by the sequence prior to the gene of interest, has affinity with Ni-affinity column and the protein of interest can be purified by imidazole elution. The pQE vectors (commercially available from Quigen) can be used for cloning the gene in three different frames such as 0, -2 and –1 frame(pQE-30, pQE-31 and pQE-32).

For this cloning, the AGT-SAL gene was first cloned in pBSKS(+) at EcoRI site (as a vector) whereas the gene was obtained from pTAdv-Sal and transformed to DH10B competent cells. The transformants were selected on LB Agar Amp(-IPTG/X-gal-) and white colonies were screened for the presence of insert using EcoRI and KpnI/SacI. The orientation of the insert was analysed using enzymes such as PstI, NcoI-SacI etc. The construct was named as pSV-SAL. From pSV-SAL, the gene was directionally cloned into pQE (pQE-30, pQE-31 and pQE-32) vectors by using AGT-SAL KpnI/SacI double digest and transformed in DH10B competent cells. The transformants were selected on LB Agar (Amp) and the transformants were screened. The recombinants were confirmed by digesting transformants plasmid with EcoRI and the three constructs were named as pExSV(1)SAL (have backbone of pQE-30), pExSV(2)SAL (have backbone of pQE-31) and pExSV(3)SAL (have backbone of pQE-32).

Further all constructs were transformed to M15 (commercially available from Quigen) competent cells for expression. M15 cells are specifically expression cells because of the presence of pREP4 which overproduces Lac repressor protein for Lac promoter and so the induction of gene of interest is tightly regulated.

The M15 cells with three constructs were grown till it reached to log phase, induction with IPTG was given and allowed for 3-4 hours. The cells were pelleted and dissolved in Tris-phosphate urea buffer(pH8.0). The samples of these were loaded to acrylamide gel with uninduced sample as control. After the protocol is standardized it will be deduced as which one of them is expressing the protein under induced conditions. The native AGT-SAL is purified. The protein was purified by Ni-NTA affinity column which has affinity for the 6X His tag and the elution was performed by buffer

containing imidazole which has higher affinity for Ni matrix and then in turn compete with 6X His tagged protein and replaces them.

The structure and function of AGT-SAL-11 was predicted using computational Biology, (Bioinformatics). Bioinformatics is a theoretical approach where predictions are carried out using computer applications; the Biological Data generated from the Laboratories till date is the source for the Databases.

Although in most cases protein production is the ultimate output for the gene protein analysis techniques are currently less suitable for high throughput screening than Nucleic Acid analysis techniques. Thus RNA analysis are the most important at present.

To find any similar pattern or similar molecules in the database a program BLAST (www.ncbi.nlm.nih.gov/BLAST) was performed but no significant results were obtained (using the gene sequence).

The subsequent tests mentioned below were performed to study the Protein level, the stage that actually determines the Function of a gene. (AAF06789.1)

The protein sequence was also subjected to similarity search initially with BLAST with BLOSUM –62 , matrix but found no interesting results. BLOSUM stands for Block Summation matrix, which is used to find molecules, which are related to one another having similar sequences and accounts for similar functions as well.

For a more specific approach the tests were extended to FASTA, (www.ebi.ac.uk) a stringent method for finding sequence similarity, in this attempt we could count on a group of hits which resembled AGT-SAL-11, using a matrix of BLOSUM-40. BLOSUM 40 is used to find distantly related molecules.

AGT-SAL-11 molecule shows similarity with Proteinase Inhibitors of the Bowman – Birk II type of super-family of Proteinase Inhibitors, which are from the following species.

Ex. *Vicia faba, Vigna sp., Glycine max (Soyabean)* .

These molecules are generally bi-functional units, which can act on two different substrates. (Substrates being Chymotrypsin , Elastase, Trypsin, subtilisin) .

These Bowman –Birk type Proteinase inhibitors including AGT-SAL-11 molecules commonly have Glycosylation sites where a carbohydrate moiety can bind, most likely carbohydrates which bind with these molecules are Mannose sugars.

The 3D Structure of the Bowman–Birk type proteinase inhibitors shows the molecules tend to have an αβ type of folding.

The Secondary structure of AGT-SAL –11 was predicted using the applications of Predict Protein server. The results obtained are as

a) The molecule shows a mixture of αβ type of secondary structure.
b) There are sites for Glycosylation and Phosphorylation (mostly O Glycosylation with Serine or Threonine residues).

Inhibitors of the Bowman Birk type are relatively small (about 70 amino acids length) and multiply cross linked with disulfide bridges. The Bowman-Birk inhibitors often display dual specificity, inhibiting both trypsin and chymotrypsin. No pattern has emerged to establish which inhibitors have protective effect and which do not. Inhibitor specificity does not appear to be the only factor, since some trypsin inhibitor are effective while others are not.

Experimental Procedures

1. Collection of plant materials.

a. Seeds of IR64 and Rasi, the two varieties of *Indica* rice chosen for the study were dehusked and good seeds were selected. These were surface sterilized using 70 % ethyl alcohol for 1 minute and 2 % sodium hypochlorite for 20 minutes. Surface sterilized seeds were washed repeatedly with sterile distilled water to remove traces of sterilizing agents.

Circular sterile filter papers were placed in autoclaved plastic petriplates and moistened with 20 ml sterile distilled water in the laminar flow hood. About 25 surface seeds were placed in each plate and the lid was covered and the plates were incubated at room temperature.

The seeds on an average took 2 days for germination. After germination the seedlings were allowed to grow for one week. The plates were constantly monitored for contamination. Since the plant material was to be used for RNA extraction, plates with any sign of contamination was discarded. Petriplates were irrigated whenever necessary.

Nine day old seedlings were used for inducing salt and dehydration stresses.

b. Induction of Salt Stress

For the induction of salt stress, the water in the petriplates containing 9 day old seedlings was replaced with 150 mM NaCl solution. One, two, four, eight and sixteen hours were collected by excising the endosperm and separting the seedling into root and shoot. The plant material was immediately frozen in liquid nitrogen and stored at –80 degres Celsius for RNA isolation later on.

c. Induction of moisture stress

Moisture stress was induced by allowing nine days old seedlings to desiccate gradually in inflated plastic bags at room temperature. Loss of weight of the seedling was constantly monitored. Plastic bags were changed frequently to decrease humidity inside the bag. When the seedlings recorded 30 % and 40 % weight loss, samples were collected by excising the endosperm and separating the seedling into root and shoot and freezing them.

d. Controls

Unstressed nine day old seedlings of Rasi and IR 64, collected in the same manner as described above, were used as controls.

II Isolation of Total RNA

a. Preparation of RNA extraction

The following precaution were taken to inhibit ribonucleases.

1. All glassware and heat resistant materials (pestle and mortar, forces etc. were baked overnight in an oven.
2. 0.1 % DEPC (diethylpyrocarbonate) was added to all solutions (except those containing Tris) incubated overnight after thorough shaking and then autoclaved.

3. All plastic ware were treated with 10 % hydrogen peroxide overnight, autoclaved and dried properly after use.
4. Clean disposable gloves were used at all stages of RNA extraction.

b. RNA Isolation

Single step method of RNA isolation by acid Guanidium thiocyanate Phenol- chloroform extraction (Sacchi et. Al 1987) was employed to isolate total RNA. The procedure consisted of following steps:

1. 0.5 to 1 gm of tissue was ground in liquid nitrogen using pestle and mortar to make a fine powder.
2. To this 6ml of freshly prepared extraction buffer was added and homogenized.
3. To the homogenate taken in a centrifuge tube, the following reagents were sequentially added and mixed thoroughly after addition of each reagent:
    a. ml of 2M sodium acetate (pH4)
    b. 10ml of phenol (Saturated with DEPC treated water)
    c. 2ml of chloroform: isoamyl alcohol (49:1) mixture

This was incubated on ice for 15minutes and centrifuged at 8000 rpm for 12 minutes at 4 degrees C. The aqueous phase was carefully transferred to a fresh centrifuge tube and 10 ml of iso-propanol was added and mixed well and incubated at –20 degrees for 1 hour. The tube was centrifuged at 14,500 rpm for 20minutes at 4 °C. The pellet was re-suspended in 3ml of extraction buffer and 3ml of iso-propanol was added, mixed well and incubated at –20 °C for 1 hour. The tube was centrifuged at 14,500 rpm for 20 minutes at 4 °C. The pellet was washed with 1ml of 75 %ethanol, centrifuged at 14,500 rpm for 15minuted at 4 °C. The pellet was dissolved in DEPC treated water and stored at - 80°C.

a. Determination of RNA concentration

3µl of RNA extract was taken in 1ml of DEPC treated water for spectrophotometric quantification and purity analysis. Absorbance at 260nm and 280nm was taken using a "spectronic Genesis-5' spectrophotometer. RNA concentaruions were determined based on the relationship that an OD of 1 at 260nm corresponds to 40µg of RNA. RNA purity was assessed by calculating the A260/280 ratios The ratio should be close to 2 for a good RNA extraction.

b. Checking of RNA integrity by Submarine Agraose Gel electrophoresis.

A 100ml 1.2 % formaldehyde agarose gel was cast by melting 1.2g of agarose (RNase free) in 73.3 ml of DEPC treated water. Just before pouring the gel, 10 ml of 10 X MOPS/EDTA and 16.7 ml of formaldehyde (2.2M) was added.

30µg of RNA was taken in 25µl of the gel loading dye mixed well and heated at 65degrees celsius for 15 minute on a dry bath and snap cooled on ice before loading on the gel.

3µl of 0.24kb to 9.5kb RNA ladder from GIBCO BRL containing a mixture of 6 synthetic poly (A) tailed RNAs (0.5µg each) of sizes 9.49kb, 7.46kb, 4.40kb, 2.37kb, 1.35kb and 0.24kb was used as a marker for these gels (Fig no.2).

Horizontal or submarine agarose gel electrophoresis system was used. IX MOPS/EDTA was used as the electrode buffer. A potential difference of 5-10 volts per cm (distance between the electrodes) was used for the anionic run.

The two prominent RNA bands of sized 4.7kb and 1.9kb correspond to 28s and 18s ribosomal RNA activity (figure no.1) Faint bands of 2.9kn (23s chloroplast rRNA) and 1.5kb (16s chloroplast rRNA) can also be visualized. 5s rRNA is about 120bp and runs faintly below the dye front. The 240bp RNA size marker comigrates with the Bromo-phenol blue dye front. The smear below the dye front also represents degraded RNA apart from tRNA and a small mRNA population. The rest of the RNA is the mRNA population. DNA(contamination) stays in the well hardly moves. A good RNA extract when runs on the gel shows minimum or no DNA in the well, distinct rRNA bands, prominent smear up to the dye front and a faint fizzy band below the dye front. (Fig No.1)

We claim:

1. An isolated nucleic acid sequence plant comprising a polynucleotide, AGT- SAL 11 having a sequence SEQ.ID No.1.

2. A nucleic acid sequence as claimed in claim 1 wherein said AGTSAL polynucleotide sequence encodes a polypeptide as shown in SEQ ID No. 2.

3. A nucleic acid sequence as claimed in claim 1 wherein said polynucleotide sequence is a full length AGTSAL 11 gene.

4. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide sequence is a complete and mature AGTSAL 11 protein.

5. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide has bi-functional units.

6. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide has glycosylation and phosphorylation sites.

7. A nucleic acid sequence as claimed in claim 2 wherein said glycosylation is O glycosylation.

8. A nucleic acid sequence as claimed in claim 2 wherein said AGT-SAL 11 has a mixture of $\alpha\beta$ type of secondary structure.

9. A nucleic acid sequence as claimed in claim 2 wherein said polypeptide has similarity with proteinase inhibitors of Bowman Birk II type of super family of proteinase inhibitors.

10. A transgenic plant comprising a recombinant expression cassette comprising a plant promoter operably linked to the polynucleotide sequence as claimed in claim 1.

11. A method for conferring salt tolerance on a plant, the method comprising introducing into the plant a recombinant expression cassette comprising a plant operator operably linked to AGT-SAL polynucleotide sequence as claimed in claim 1.

SEQUENCE LISTINGS

SEQUENCE ID NO. 1

BASE COUNT 163 a 145 c 170 g 195 t
ORIGIN

```
        1 tttaccttgc ctgctcggat ggcagcaaac tccatcttgg ggtgtggcgt gagcacacca
       61 agaaattctc ccctcagtgg tttgcagctg tccatgccgc tgttccattc attggaatgc
      121 tgaggaaatc tgtcaacatg cccaagactg ccatggcatt caccatagca gcctccattg
      181 ttggtcagac aatcgggtcg agggcggagc gcattcgtct gaaggcactg gctgcaaaga
      241 gcgacgctga ttccaccacc gtggctgaca tgtatccaaa caagactgca aattgcagtg
      301 acaccgaggg caaggcatgg gatccgctcg cgatgaagat gatggcggga cgggcttctg
      361 gtggtgctgc tgctccaaca ccaagcatgt gtttctgatt gctcactgat tggaaaattt
      421 gtatctacca gtatccctgg agagtggaga gttgatattg agtctatttt atcttgtgat
      481 gtaattgcct ttgcttgtcc ctcagaagta ttcgtttgtt tgtgggatga gacaagtgga
      541 ataagagtgc tactatatac acgatcattc tgttgttaag tttgccagtt ctgcagttca
      601 tgtatctgta atttgatgat gctggatttc tactatttat caatcgtcat tatactgtgt
      661 gtaaaaaaaa aaa
```

SEQUENCE ID NO. 2:

```
MVDTNFPISEQSETHAWCWSSSTTRSPSRHHLHRERIPCLALGV
TAICSLVWIHVSHGGGISVALCSQCLQTNALRPRPDCLTNNGGCYGECHGSLGHVDRF
PQHSNEWNSGMDSCKPLRGEFLGVLTPHPKMEFAAIRAGKV
```

12. An isolated nucleic acid sequence plant substantially as herein described with reference to the accompanying drawings.

13. A transgenic plant substantially as herein described with reference to the accompanying drawings.

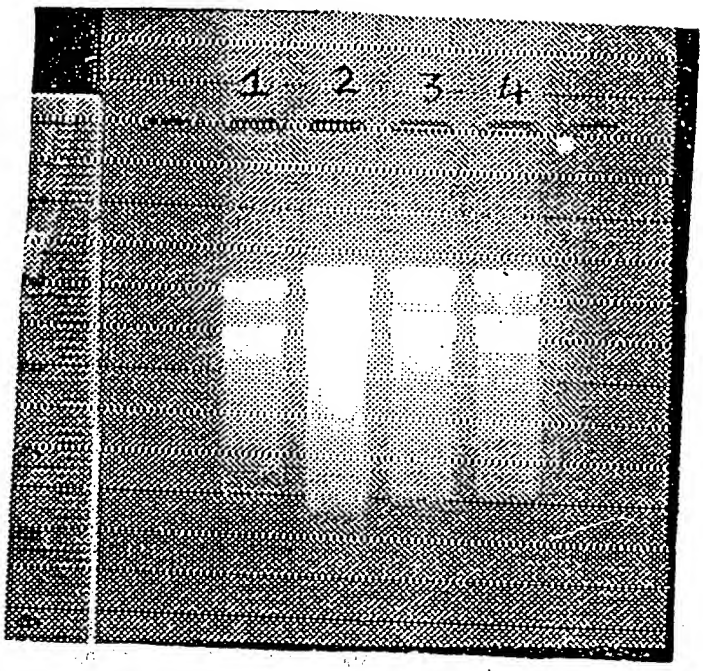
14. A method for conferring salt tolerance on a plant substantially as herein described with reference to the accompanying drawings.

Dated this 10th day of October, 2000

of **Anand & Anand Advocates**
ATTORNEY FOR THE APPLICANT

ABSTRACT

The present invention relates to an isolated nucleic acid sequence AGT-SAL 11 encoding polypeptides which confers salt tolerance on plants and other organisms.



Lanes : 1 2 3 4

IRG4 S.S IRG4C RR51 S.S RR51 C
IOHL IOHL IOHL IOHL

Fig. 1

Thanks

of Anand And Anand Advocates
Attorney For The Applicant

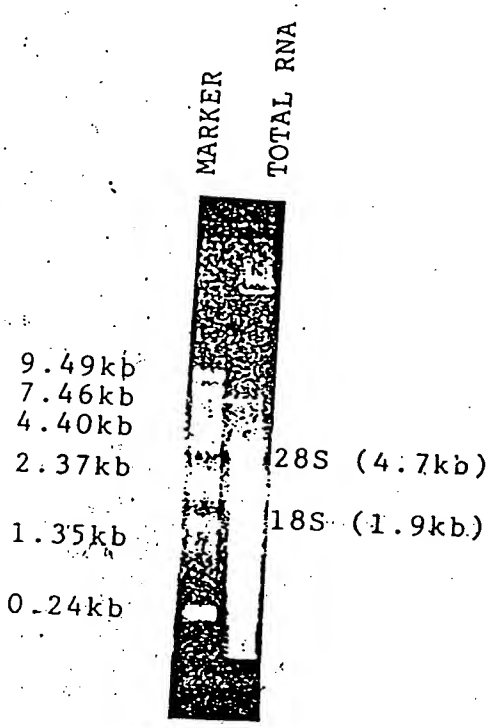


Fig. No. 1 Sample RNA agarose (denaturing gel) with size markers.

of Anand And Anand Advocates
Attorney for The Applicant

09/668025
SCO

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
11345(PCT)		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IN 00/00099	11/10/2000	13/10/1999
Applicant		
AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IN 00/00099

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/415 C07K14/81 C12N15/82

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PATELL V.M. ET AL.: "Oryza sativa Variety IR64 (cDNA clone AGTSAL-11 from 7 days old seedlings)" EMBL DATABASE ENTRY AF192975; ACCESSION NO. AF192975, 9 November 1999 (1999-11-09), XP002169663 cited in the application	1-10
A	MOONS A. ET AL.: "MOLECULAR AND PHYSIOLOGICAL RESPONSES TO ABSCISIC ACID AND SALTS IN ROOTS OF SALT-SENSITIVE AND SALT-TOLERANT INDICA RICE VARIETIES" PLANT PHYSIOLOGY, vol. 107, 1995, pages 177-186, XP000983692 ISSN: 0032-0889 page 180, column 2, paragraph 3 -page 184, column 1, paragraph 3	1-11

-/--

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2001	28/06/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schönwasser, D

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BAEK J.-M. ET AL.: "Nucleotide sequence homology of the cDNAs encoding soybean Bowman-Birk type proteinase inhibitor and its isoinhibitors" BIOSCIENCE, BIOTECHNOLOGY AND BIOCHEMISTRY, vol. 58, no. 5, 1994, pages 843-846, XP002123337 ISSN: 0916-8451 the whole document	9

1

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
ANAND & ANAND, Advocates
Attn. Anand, Pravin
B-41, Nizamuddin East
New Delhi - 110 013
INDIA

Applicant's or agent's file reference 11345(PCT)	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/IN 00/00099	International filing date *(day/month/year)* 11/10/2000
Applicant AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD.	

Date of mailing *(day/month/year)* 28/06/2001

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later):

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Renate Jordan

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

(60)

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

ANAND & ANAND

ANAND, Pravin
Anand and Anand, Advocates
B-41, Nizamuddin East
New Dehli 110 013
INDE

13 AUG 2001

RECEIVED

Date of mailing (day/month/year) 03 August 2001 (03.08.01)	
Applicant's or agent's file reference 11345(PCT)	**IMPORTANT NOTIFICATION**
International application No. PCT/IN00/00099	**International filing date** (day/month/year) 11 October 2000 (11.10.00)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
AVESTHAGEN GRAINE TECHNOLOGIES PVT. LTD. "Sunbeam" 106 Prenderghast Road Secunderabad - 500 003 Andhra Pradesh India	IN	IN
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
AVESTHA GENGRAINE TECHNOLOGIES PVT. LTD. "Discoverer", 9th floor Unit 3, International Tech Park Whitefield Road Banglore 560 066 India	IN	IN
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004192498

PATENT COOPERATION TREATY

(61)

PCT

From the INTERNATIONAL BUREAU

To:

ANAND & ANAND

13 AUG 2001

RECEIVED

ANAND, Pravin
Anand and Anand, Advocates
B-41, Nizamuddin East
New Dehli 110 013
INDE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year)	
03 August 2001 (03.08.01)	
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
11345(PCT)	
International application No.	**International filing date** (day/month/year)
PCT/IN00/00099	11 October 2000 (11.10.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
PATELL, Villo, Morawala ANTONY, Chettoor Mathai CHANDRAN, Divya MADURAPPA, Ashok	IN	IN
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
"Discoverer", 9th Floor Unit 3, International Tech Park Whitefield Road Banglore - 560 066 Karnataka India	IN	IN
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

004192507